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13. ABSTRACT (Maximum 200 Words)

Prostate cancer is the most common non-cutaneous malignancy in men. Radiation therapy is a common treatment for this disease however, most patients receive a similar dose of radiation (70-76 Gy) regarless of individual clinical, pathological, or molecular characteristics of the tumor. The hypothesis of this project is that all prostate carcinomas are unique and that by identifying specific tumor markers or other molecular characteristics using our dynamic tissue culture system (Parrish et al, 2002), we can identify those tumors most sensitive to radiation therapy. The specific aims for the first year were to use prostate biopsy tissue, obtained retrospectively, and adapt our organ culture technique to the requirements of prostate biopsy specimens. We have been able to determine the optimal biopsy core size and tissue culture medial conditions. We have also demonstrated that basal cells present in the prostate glandular tissue proliferated over the 72 hour time period of organ culture. We have maximized the length of time that tissue remains viable in our dynamic tissue culture system.

We are now ready to begin Aim II of the proposal determining the baseline readiosensitivity of prostate tissue and assessing the roles of p53, bcl-2, and NFKB in the intrinsic radiosensitivity of prostate tissue. We hope to further profile these biomarkers and using them to predict prostate tissue radiosensitivity will aid in the diagnosis and prognosis of this significant cancer.

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INTRODUCTION

Prostate cancer is the most common non-cutaneous malignancy in men. Radiation therapy is a common treatment for this disease however, most patients receive a similar dose of radiation (70-76 Gy) regardless of individual clinical, pathological, or molecular characteristics of the tumor. The hypothesis of this project is the concept that all prostate carcinomas are unique and that by identifying specific tumor markers or other molecular characteristics using our dynamic tissue culture system (Parrish et al, 2002), we can identify those tumors most sensitive to radiation therapy. The specific aims for the first year were to adapt our organ culture technique to the requirements of prostate biopsy specimens. We have been able to determine the optimal biopsy core size and tissue culture media conditions. We have also maximized the length of time that tissue remains viable in our dynamic tissue culture system.

BODY

Introduction

We have previously demonstrated through the production of very thin (275-300 μm) prostate tissue slices, which are highly reproducible, and the use of a dynamic organ culture system in which the tissue is not continuously submerged, that our precision-cut tissue slice system represents an advance over traditional prostate organ culture. Given the retention of stromal-epithelial interactions, the ability to investigate zone-specific features, and the maintenance of cellular viability and function for several days, prostate slices represent a unique *in vitro* model to investigate prostate cellular proliferation and cytotoxicity (Parrish et al, 2002).

Materials and Methods

The dynamic organ culture incubator, titanium rollers, and slice inserts were purchased from Vitron, Inc. (Tucson, AZ). Keratinocyte growth media (K-SFM) and supplements were obtained from Sigma Chemical Co. (St. Louis, MO), as was the lactate dehydrogenase assay system (kit # 340-LD). Fetal bovine serum (FBS) was purchased from Gibco (Grand Island, NY). The ELISA system for the detection of PSA was purchased from Alpha Diagnostics (San Antonio, TX). The proliferation marker MIB-1 (reactive with Ki-67) was obtained from Beckman Coulter (Fullerton, CA). All primary antibodies were detected using biotinylated secondary antibodies followed by a streptavidin-peroxidase conjugate and DAB chromogen with an automated system obtained from Ventana Medical Systems (Tucson, AZ).

Human Prostate Tissue

The University of Arizona Institutional Review Board approved the protocol for the procurement of human tissue, obtained from prostate biopsy. During brachytherapy, the prostate gland was identified using transrectal ultrasound and biopsy was guided by C-arm fluoroscopy. Transperineal biopsy specimens (3-6) were obtained using 18G MaxCore® Disposable Biopsy Units (C.R. Bard, Inc., Murray Hill, N.J.). There were a total of 11 prostates sampled for this study out of a total of 14 patients who signed consent forms.

Biopsy cores

Each biopsy core was approximately 8-10 mm in length with a diameter of 0.0126 mm or 12.6 μm. The cores were placed onto titanium roller inserts that were blotted and loaded into glass scintillation vials containing 1.7 ml of media.

Dynamic Organ Culture System

Keratinocyte serum free media (KSFM) supplemented with (10% v/v) fetal bovine serum (FBS) was the media used in all studies. The media also contained antibiotics, 50 units/ml of Gentamicin and 100 units/ml Penicillin G/ Streptomycin, and 0.625 μg/ml Fungizone an antifungal drug. The scintillation vials were closed with a cap, which has a 2 mm central opening, that allows for gas exchange. Vials were then placed in the dynamic organ culture incubator that was gassed with 95%O_2:5%CO_2 at a flow rate of 1ml/minute. The incubator temperature was a constant 37°C. The media was changed every 24 hours. The dynamic organ culture incubator is unique in that it rotates the scintillation vials allowing the biopsy core on the titanium rollers to be alternately submerged in media and then exposed to the ambient gases (Parrish et al, 1995). This is different from traditional submersion culture techniques used in prostate explant culture and affords much better exchange of nutrients, wastes, and gases.

Tissue Preparation

Three to six punch biopsy specimens were collected during the prostate brachytherapy procedure. All biopsy specimens were immediately placed in chilled, sterile normal saline solution. The first two biopsy specimens were processed *en bloc* for thin section slide preparation. Each specimen was fixed in 10% formalin and then embedded in paraffin. The specimens were sectioned via microtome (5 μm) and then stained. Stains used included H&E for routine pathologic evaluation. Other immunohistochemistry stains include PSA, a measure of normal prostate tissue secretory activity and Ki-67, a nuclear stain for mitotic or proliferative activity. Twenty-four hours after the biopsy, selected specimens were irradiated with various doses of ionizing radiation using a ^{60}Co irradiator. At specific time intervals after irradiation, the specimens were removed from the organ culture incubator and fixed in formalin for subsequent staining. Specimens were stained similarly to the baseline tissues noted above.

The biopsy specimens were labeled as follows: the format will be HPBx-*xy*.

Where HPBx will indicate the Human Prostate Biopsy Study, *x* represents the patient number, *y* represents the sample identifier (total incubation period, whether or not the sample was irradiated, period of incubation after radiotherapy (RT). Note: All irradiation was performed approximately 24 hours after initial biopsy collection. Total incubation time was the incubation period prior to RT plus the incubation period after RT. All Controls will be matched to this total incubation time, so as to eliminate potential changes that may occur simply due to different incubation periods.

Sample Identifier (y)	Meaning (Total incubation period, RT dose, Incubation Period after RT)
1	Control: Incubation for hours (2-4 hrs), No RT
2	Control: Incubation for 24 hours, No RT
3	24 hours, 10 Gy, 1-2 hours after RT
4	Control: 48 hours, no RT
5	48 hours, 10 Gy, incubated for 24 hours after RT
6	Control: 72 hours, No RT
7	72 hours, 10 Gy, incubated for 48 hours after RT

Example: HPBx 2-5 would mean: Human Prostate Biopsy patient number 2, the specimen was incubated for 24 hours prior to RT, received 10 Gy RT, and then was incubated for an additional 24 hours prior to formalin fixation (Total incubation time = 48 hrs)

Glands were graded as negative (glands with PSA expression), luminal (glands with positive PSA staining of luminal cells but negative basal cell expression), or full thickness (abnormal PSA staining, with all cells showing expression). The type of staining for each gland was determined for each slice and expressed as the mean ± standard deviation. The number of Ki-67 immunoreactive nuclei was expressed as the mean value x 100 ± standard error.

Results

Prostate biopsy cores were obtained from a total of 11 patients. 19 patients were consented but specimens were not collected due to technical difficulties-4, withdrew consent-3, and one had previous radiation. These cores were approximately 5 mm in length and the diameter was analogous to the internal diameter of an 18G needle. We attempted to obtain 3 cores from each patient. Then each core could be

cut in half and there would be enough tissue for 6 treatment/time periods. However, not all specimens were adequate due to sampling errors, tissue damage, presence of frank carcinoma, acute and/or chronic prostatitis, to name a few of the difficulties encountered. As a result there was not always enough tissue for each category listed (Table 1).

Histological evaluation.

The initial experiments were performed to investigate the maintenance of organ architecture and cellular heterogeneity of prostate biopsy cores. Histological evaluation of the biopsy cores revealed maintenance of the normal glandular structure of the organ following slicing (Fig. 1-4), suggesting that the slicing process was not associated with dramatic damage. The architecture of the prostate was maintained in cores incubated for up to 72 hours. Although a gradual loss of luminal cells was observed throughout the culture, the basal cell population was not only maintained in the presence of FBS but also proliferated from days 1-3. Ki-67 staining on the other hand, although present, was inconsistent and not comparable to the degree of staining expected with a high degree of proliferation (Fig. 5). There was no evidence of central tissue necrosis usually associated with inadequate penetration of media into the biopsy core.

Key Research Accomplishments

- Determined the optimal prostate biopsy core size for maintaining biopsy specimens in the dynamic tissue culture system. Dimensions of the cores are analogous to the inner diameter of an 18G needle and approximately 3-4 mm in length.
- Determined the optimal tissue culture media conditions. Keratinocyte serum-free media (KSFM) with 10% (vol./vol.) fetal calf serum. The media also contained antibiotics, 50 units/ml of Gentamicin and 100 units/ml Penicillin G/ Streptomycin, and 0.625 μg/ml Fungizone an antifungal drug. Media was changed every 24 hours.
- Determined that prostate biopsy cores can maintain viability up to 72 hours. Tissue architecture was normal, there were no areas of central necrosis, and basal cell proliferation was maximal at 72 hours of incubation.

Reportable Outcomes

The results of this type of work have been previously reported (Parrish et al, 2002).

Conclusions

We have completed the goals outlined in Aim I. We are now ready to submit a new consent form for the purpose of enrolling new patients with prostate cancer. We believe that the goals of Aim II are attainable and we need to begin enrolling patients right away. The original goal of consenting a total of 25 subjects for Aims I and II will need to be revised. The 11 patients from the retrospective group were to expected to provide adequate samples to complete Aim I and then supply further specimens along with another 14 patients to complete Aim II. The total of 25 patients would allow a correlation coefficient of 0.55 with 80% power.

There are no samples left to begin Aim II and we will quickly resubmit our previously approved consent form with the necessary changes. We will be meeting soon with our biostatisticians to assess our new goals as well as to reevaluate the original power calculations. We have a new radiation oncologist who is interested in collaborating with us.

References

Parrish AR, Sallam K, Nyman DW, Orozco J, Cress AE, Dalkin BL, Nagle RB, and Gandolfi AJ. Culturing precision-cut human prostate slices as an in vitro model of prostate pathobiology. Cell Biology and Toxicology. 18:205-219, 2002.

Appendix

Figures 1-5

Table 1

Reprint of *Cell Biology and Toxicology* manuscript

Figures Legend

Prostate biopsy tissue was placed into a dynamic organ culture system in keratinocyte serum-free media supplemented with 10% FBS (fetal calf serum) and maintained for up to 72 hour. Biopsy tissue was fixed in formalin and paraffin-embedded. Thin sections (5-7μm) were stained with hematoxylin and eosin or Ki-67.

Figure 1 HPBx 24. Prostate biopsy tissue (patient #2, control) was incubated for 48 hours. Prostate glandular tissue viable. Note robust basal cell proliferation and preservation of luminal cells. Magnification 40X.

Figure 2 HPBx 25. Prostate biopsy tissue (patient #2, radiation 10Gy) incubated for 48 hours. Tissue is remarkable for the presence of carcinoma. Glands are poorly differentiated and basal cells are not proliferative. Magnification 40X.

Figure 3 HPBx 54. Biopsy tissue (patient #5, control) incubated 48 hours. Prostate glandular tissue viable. Note robust basal cell proliferation and preservation of luminal cells. Magnification 40X.

Figure 4 HPBx 57. Biopsy tissue (patient #5, radiation 10Gy) incubated for 72 hours. Note basal cell proliferation. Magnification 40X.

Figure 5 HPBx 106. Prostate biopsy tissue (patient #10, control) incubated for 72 hours. Tissue is treated to react immunohistochemically to Ki-67. Staining indicates the presence of actively replicating DNA, thus a different measure of cell proliferation. Magnification 80X.

Figure 1. HPBx 24

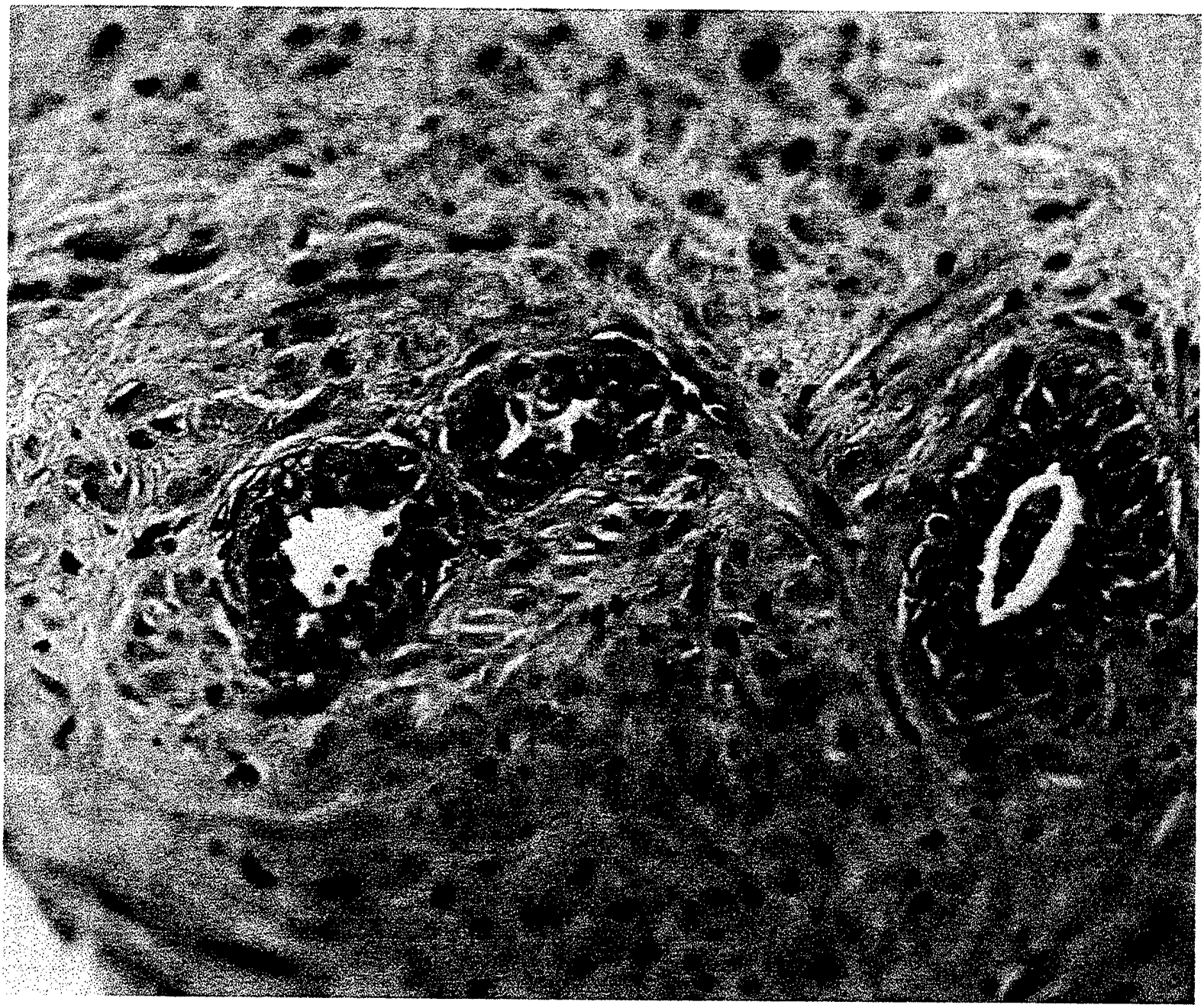

Figure 2. HPBx 25

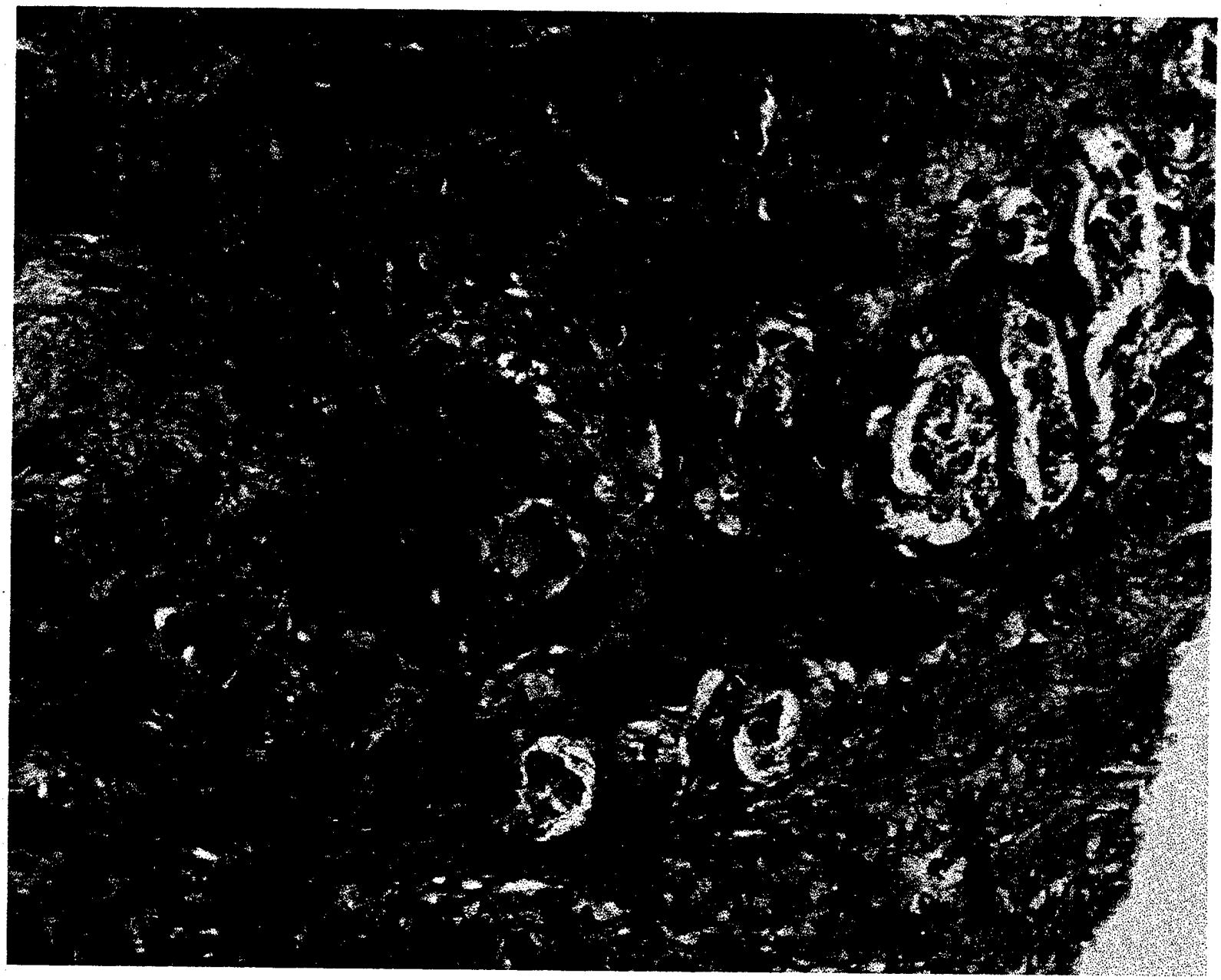

Figure 3. HPBx 54

Figure 4. HPBx 57

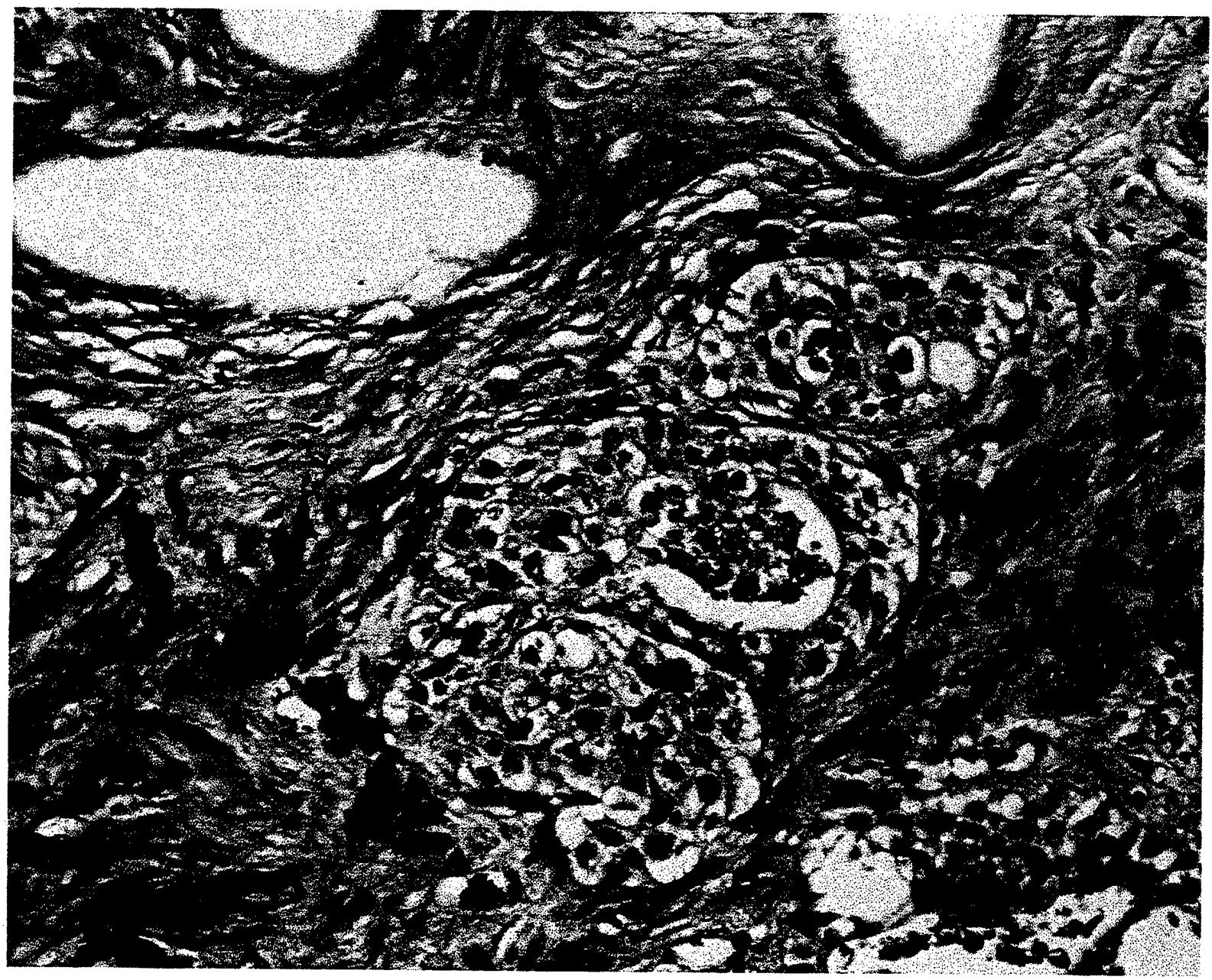

Figure 5. HPBx 106

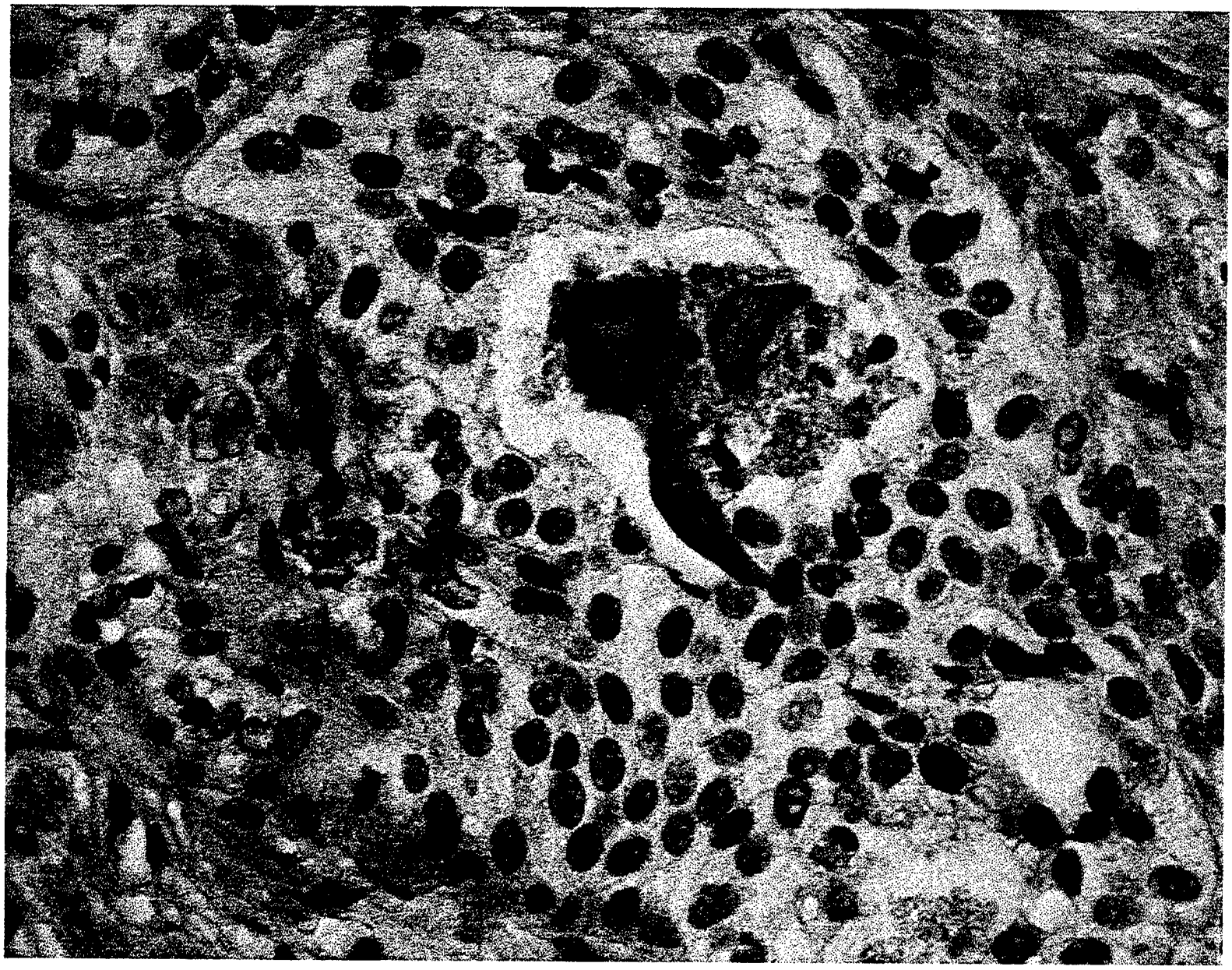

Table 1 **Human Prostate Biopsy Study**

ID	Tx	H&E	Ki-67	Notes
HPBx-11	C: 2-4 hr	X	X	H&E: no basal cell proliferation KI-67: negative
HPBx-12	C: 24hr	X	X	H&E: basal cell proliferation + KI-67: negative
HPBx-13	24h 10 Gy			
HPBx-14	C: 48hr	X	X	H&E: basal cell proliferation + KI-67: negative
HPBx-15	48h 10 Gy			
HPBx-16	C: 72hr			
HPBx-17	72h 10 Gy			
ID	**Tx**	**H&E**	**Ki-67**	**Notes**
HPBx-21	C: 2-4 hr			
HPBx-22	C: 24hr			
HPBx-23	24h 10 Gy			
HPBx-24	C: 48hr	X	X	H&E: basal cell proliferation + Ki-67: negative
HPBx-25	48h 10 Gy	X	X	H&E: no proliferation Ki-67: negative
HPBx-26	C: 72hr	X	X	H&E: no proliferation Ki-67: negative
HPBx-27	72h 10 Gy	X	X	H&E: basal cell proliferation + Ki-67: negative

ID	**Tx**	**H&E**	**Ki-67**	**Notes**
HPBx-31	C: 2-4 hr			
HPBx-32	C: 24hr			
HPBx-33	24h 10 Gy			
HPBx-34	C: 48hr	X	X	H&E: no proliferation Ki-67: negative
HPBx-35	48h 10 Gy			
HPBx-36	C: 72hr			
HPBx-37	72h 10 Gy			
ID	**Tx**	**H&E**	**Ki-67**	**Notes**
HPBx-41	C: 2-4 hr			
HPBx-42	C: 24hr	X	X	H&E: basal cell proliferation + Ki-67: negative
HPBx-43	24h 10 Gy			
HPBx-44	C: 48hr	X	X	Poor quality specimen
HPBx-45	48h 10 Gy	X	X	H&E: basal cell proliferation + Ki-67: negative
HPBx-46	C: 72hr			
HPBx-47	72h 10 Gy			

ID	**Tx**	**H&E**	**Ki-67**	**Notes**
HPBx-51	C: 2-4 hr			
HPBx-52	C: 24hr	X	X	H&E: basal cell proliferation + Ki-67: negative
HPBx-53	24h 10 Gy			
HPBx-54	C: 48hr	X	X	H&E: basal cell proliferation + Ki-67: negative
HPBx-55	48h 10 Gy	X	X	H&E: basal cell proliferation + Ki-67: negative
HPBx-56	C: 72hr	X	X	H&E: + but no proliferation Ki-67: negative
HPBx-57	72h 10 Gy			H&E: basal cell proliferation + Ki-67: negative
HPBx-57	72h 10 Gy	X	X	H&E: basal cell hyperplasia
ID	**Tx**	**H&E**	**Ki-67**	**Notes**
HPBx-61	C: 2-4 hr	X	X	H&E: basal cell proliferation + Ki-67: negative
HPBx-62	C: 24hr	X	X	H&E: basal cell proliferation + Ki-67: negative
HPBx-63	24h 10 Gy	X	X	H&E: + but no proliferation Ki-67: negative
HPBx-64	C: 48hr	X	X	H&E: basal cell proliferation + Ki-67: negative
HPBx-65	48h 10 Gy			
HPBx-66	C: 72hr			
HPBx-67	72h 10 Gy			

ID	**Tx**	**H&E**	**Ki-67**	**Notes**
HPBx-71	C: 2-4 hr			
HPBx-72	C: 24hr			
HPBx-73	24h 10 Gy			
HPBx-74	C: 48hr			
HPBx-75	48h 10 Gy			
HPBx-76	C: 72hr	X	X	H&E: basal cell **highly** proliferative /Ki-67: negative
HPBx-77	72h 10 Gy			
ID	**Tx**	**H&E**	**Ki-67**	**Notes**
HPBx-81	C: 2-4 hr	X	X	poor quality specimen
HPBx-82	C: 24hr	X		H&E: basal cell proliferation +
HPBx-83	24h 10 Gy	X		H&E: basal cell proliferation +
HPBx-84	C: 48hr			
HPBx-85	48h 10 Gy			
HPBx-86	C: 72hr			
HPBx-87	72h 10 Gy			

ID	**Tx**	**H&E**	**Ki-67**	**Notes**
HPBx-91	C: 2-4 hr			H&E: basal cell proliferation + Ki-67: negative
HPBx-92	C: 24hr			H&E: basal cell proliferation + Ki-67: negative
HPBx-93	24h 10 Gy			H&E: basal cell proliferation + Ki-67: 20%
HPBx-94	C: 48hr			
HPBx-95	48h 10 Gy			
HPBx-96	C: 72hr			H&E: basal cell proliferation + Ki-67: negative
HPBx-97	72h 10 Gy			
ID	**Tx**	**H&E**	**Ki-67**	**Notes**
HPBx-101	C: 2-4 hr			
HPBx-102	C: 24hr			H&E: basal cell proliferation negative/Ki-67: negative
HPBx-103	24h 10 Gy			
HPBx-104	C: 48hr			
HPBx-105	48h 10 Gy			
HPBx-106	C: 72hr			H&E: basal cell proliferation + Ki-67: 20% pos.
HPBx-107	72h 10 Gy			

ID	**Tx**	**H&E**	**Ki-67**	**Notes**
HPBx-111	C: 2-4 hr			
HPBx-112	C: 24hr			
HPBx-113	24h 10 Gy			
HPBx-114	C: 48hr			
HPBx-115	48h 10 Gy			
HPBx-116	C: 72hr			H&E: basal cell proliferation + Ki-67: negative
HPBx-117	72h 10 Gy			
ID	**Tx**	**H&E**	**Ki-67**	**Notes**
HPBx-121	C: 2-4 hr			
HPBx-122	C: 24hr			
HPBx-123	24h 10 Gy			
HPBx-124	C: 48hr			
HPBx-125	48h 10 Gy			
HPBx-126	C: 72hr			
HPBx-127	72h 10 Gy			

Cell Biology and Toxicology. 2002; 18: 205–219.

Culturing precision-cut human prostate slices as an *in vitro* model of prostate pathobiology

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Keywords: prostate, dynamic organ culture system, peripheral, transitional, stromal–epithelial

Abstract

Due to the complex morphology of the prostate, it was hypothesized that precision-cut tissue slices from human prostate would provide a unique *in vitro* model. Precision-cut slices were generated from zones of human prostate and their viability was assessed under conditions of different media for up to 120 h. Slices were also exposed to several concentrations of $CdCl_2$, which was used as a model toxicant. Maintenance of both stromal and epithelial cells was noted; however, there was a gradual loss of luminal epithelial cells when the medium was not supplemented with dihydrotestosterone (DHT). Minimal leakage of lactate dehydrogenase occurred throughout the incubation. Prostate-specific antigen (PSA) was detected in the medium at all time points, although the rates of secretion fell over time. There was a loss of PSA-positive cells when the medium was not supplemented with DHT, consistent with a loss of luminal cells, whereas PSA-positive cells were maintained in the DHT-supplemented media. A proliferation of basal cells was observed in the presence of media containing 10% fetal bovine serum. Exposure of slices to $CdCl_2$ demonstrated a dose–response effect ranging from proliferation to complete cellular necrosis. Given the retention of stromal–epithelial interactions and the use of acquired human tissue, prostate slices represent a unique *in vitro* model for investigating human prostate pathobiology.

Introduction

Prostate diseases, both benign and malignant, are a significant cause of morbidity and mortality (Mettlin et al., 1998). Understanding of the molecular mechanisms underlying prostate pathobiology is complicated by the heterogeneity of the prostate at both the organ and cellular levels. The prostate consists of three anatomic zones – transitional, central, and peripheral – that contain glands and ducts lined by secretory and basal cells (epithelial compartment) within a complex network of stromal cells (mesenchymal cells). The peripheral zone is the major site of origin of adenocarcinoma, while the transitional zone is the site of benign prostatic hyperplasia (McNeal, 1969, 1988). Given these regional differences in disease development, it is thought that prostatic hyperplasia does not lead to cancer; however, prostatic intraepithelial neoplasia within the peripheral zone may, in fact, be the

precursor to adenocarcinomas (Montironi et al., 1996). The central zone surrounds the ejaculatory duct and is not a major site of prostatic disease or pathology.

It is well established that interactions between the stromal and epithelial components are key to the normal functions of the prostate, and may also play a role in the development of cancer (Cunha, 1994; Cunha et al., 1996; Hayward et al., 1997). In addition, considerable differences exist between the epithelial cell types in the prostate. Luminal cells of the gland are secretory, expressing cytokeratins 8 and 18 and variable amounts of 19, androgen receptor, and prostate-specific antigen (Leong et al., 1988; Ruizeveld de Winter et al., 1994). In addition, androgens are required for the maintenance of differentiated function (Cunha et al., 1987). Basal cells express cytokeratins 5, 6, 8, 10, 13, 14, and 18 (Gown and Vogel, 1984). However, they do not express PSA (Howat et al., 1988) and express the androgen receptor only sporadically (Chodak et al., 1992; Bonkhoff and Remberger, 1993). Because of the loss of epithelial subpopulations and the subsequent loss of normal stromal–epithelial interactions, the study of isolated cell lines may not be entirely representative of an integrated organ response.

Unlike for many other diseases, there are a limited number of animal models for studying prostatic cellular proliferation or cytotoxicity, severely limiting understanding of the molecular mechanisms underlying prostate pathobiology. The currently available models include tumor xenografts (McCandless et al., 1997), prostate explants (Pretlow, 1995), transgenic mouse models of prostatic carcinogenesis (Greenberg et al., 1995; Kasper, 1998), and the Dunning rat model; and larger animals such as dogs and monkeys have been studied (Maitland, 2001). Given these factors of organ heterogeneity, cellular heterogeneity, and limited availability of animal models, the development of a system using human tissue that maintains organ architecture and cellular heterogeneity for several days would be a major advance in an *in vitro* model.

Prostate tissue slices have been studied in one form or another over the past 25 years. Prostate tissue (human, dog, and rat) in slices (500 μm to 1 mm thick) or cubes (2 mm^3) has been placed in various types of incubation baths and studied for periods up to 12 h (Dube et al., 1983; Farnsworth, 1977; Koenig et al., 1977). These models differ significantly from ours, both in the degree of precision with which the slices are cut and in the incubation time.

A later study describes the use of prostate tissue slices in a culture medium (Hampton and Selman, 1992). Evaluation of this study shows that the prostate tissue used was obtained after a transplantable prostate adenocarcinoma (R3327-AT) was grafted onto the flanks of rats. The tumor was harvested and then precision-cut slices were prepared. These slices are from tumor tissue, not prostate. The incubation was limited to 6 h.

A more recent investigation has reported the use of 1 mm cubes of human prostate tissue continuously submerged in organ culture (Varani et al., 1999). This study found the cubes to retain viability for 8–20 days, with retention of tissue architecture. This organ culture approach has also been used in our laboratories. Over the past 15 years our laboratories have been developing precision-cut tissue slices as an *in vitro* model that can be used with either animal or human tissue. Culturing of precision-cut slices provides a unique *in vitro* system based on the retention of organ architecture and cellular heterogeneity/interactions. The development of automated equipment to produce very thin (275–300 μm) slices of highly reproducible dimensions and of a dynamic organ culture system was the basis for a resurgence in the use of slices as an *in vitro* model.

The applicability of precision-cut slices for pharmacological and toxicological investigations has been reviewed (Parrish et al., 1995).

The technique has been particularly useful in identifying cell-specific responses within the context of another heterogeneous organ, the kidney (Ruegg et al., 1987; Wolfgang et al., 1989). The present study was designed to use human prostate tissue that was sliced within 30 min of its surgical removal and then cultured for several days in a dynamic organ culture.

Materials and methods

Materials

The Brendel-Vitron tissue slicer, dynamic organ culture incubator, and titanium slice inserts were purchased from Vitron, Inc. (Tucson, AZ, USA). Keratinocyte growth media and supplements were obtained from Sigma Chemical Co. (St. Louis, MO, USA), as was the lactate dehydrogenase assay system (kit #340-LD). Fetal bovine serum was purchased from Gibco (Grand Island, NY, USA). The ELISA system for the detection of PSA was purchased from Alpha Diagnostics (San Antonio, TX, USA). The basal cell-specific murine monoclonal antibody KA-2 (specific for cytokeratins 5 and 14) was produced in our laboratories; the PSA antibody was obtained from Dako (Carpinteria, CA, USA). The proliferation marker MIB-1 (reactive with Ki-67) was obtained from Beckman Coulter (Fullerton, CA, USA). All primary antibodies were detected using biotinylated secondary antibodies followed by a streptavidin–peroxidase conjugate and DAB chromogen with an automated system obtained from Ventana Medical Systems (Tucson, AZ, USA). Androgen supplements were obtained from Sigma.

Human prostate tissue

The University of Arizona Institutional Review Board approved the protocol for the culturing of human tissue obtained from radical prostatectomies. The apex of the prostate was removed and a 1.5 cm slice was made through the gland in an anterior–posterior plane. A sharpended stainless steel dermatome was rotated through the organ to obtain cores of 1.5 cm length (6 mm diameter) from the peripheral and transitional zones without disturbing the margins. Attempts were made to avoid visually detectable carcinoma. Following harvest of the cores, the surface of the prostate was covered with India ink and all remaining tissue was embedded for pathologic diagnosis.

A total of 64 prostates were sampled for this study. Nine prostates were not used due to contamination, which was probably caused by chronic prostatitis.

Precision-cut slices

Each end (2 mm) of the tissue core was trimmed to provide a flat surface. The cores were cut into 5–7 mm sections and precision-cut slices (275–300 μm thick) were generated using the Brendel-Vitron tissue slicer (Parrish et al., 1995). The slices were placed onto titanium roller culture inserts that were blotted and loaded into glass scintillation vials containing 1.7 ml of medium. Approximately 1545 slices were utilized in this study. Of those slices, 868 were from the transitional zone and 577 were peripheral.

Three different variations of medium was compared: (a) keratinocyte medium supplement (bovine pituitary extract, bovine insulin, hydrocortisone, and bovine transferrin and human epidermal growth factor (EGF)) added to keratinocyte basal medium in a 1:50 ratio; (b) keratinocyte basal medium supplemented with (10% v/v) fetal bovine serum (FBS); and (c) EGF-supplemented keratinocyte basal medium supplemented with 30 mmol/L dihydrotestosterone (DHT) to assess the ability of androgen to maintain slice viability. All three medium variations contained 50 units/ml of gentamicin, 100 units/ml penicillin G/streptomycin, and 0.625 μg/ml fungizone.

The scintillation vials were closed with a cap with a 2 mm central opening, and placed in the dynamic organ culture incubator, which was gassed with 95% O_2:5% CO_2 at a flow rate of 1 ml/min. The incubator temperature was a constant 37°C. The medium was changed every 24 h. The dynamic organ culture incubator is unique in that it rotates the scintillation vials, causing the slices on the titanium rollers to be alternately submerged and then exposed to the ambient gases (Parrish et al., 1995). This differs from traditional submersion culture techniques used in recent prostate explant culture and affords much better exchange of nutrients, wastes, and gases.

$CdCl_2$ exposure

Prostate tissue slices were also incubated for 1–3 days at $CdCl_2$ concentrations of 10 μmol/L, 100 μmol/L, 500 μmol/L, and 1 mmol/L. Slices were removed at 24 h intervals and were processed for histopathology using hematoxylin and eosin (H&E) and Ki-67 staining. Immunohistopathology staining for PSA was also performed.

Tissue viability

Histological evaluation. Following incubation for up to 120 h, slices were collected and fixed in 10% neutral buffered formalin. Tissue sections were paraffin embedded, sectioned (6 μm), and placed onto charged slides. Following paraffin removal, sections were stained with H&E, and histological evaluation was performed. Additional sections were evaluated by immunohistochemical procedures to evaluate PSA, basal cell specific cytokeratin expression, and Ki-67 nuclear reactivity.

Lactate dehydrogenase assay system. LDH activity was determined using a commercially available kit from Sigma Chemical Co. Briefly, 200 μl of culture medium was added to 0.5 ml reagent and the absorbance at 340 nm was read at 30 s intervals over 5 min. The changes in absorbance were used to determine LDH activity (U/L). Units of LDH activity are defined as the amount of enzyme that will catalyze the formation of one micromole of NADH per minute.

Detection of PSA secretion. Two μl of culture medium was placed in the wells of a 96-well plate coated with mouse anti-human PSA. One hundred μl of anti-human PSA horseradish peroxidase was added to each well for 30 min prior to colorimetric detection at 450 nm. Values were determined by regression analysis using a standard curve (0–60 ng/ml).

Immunohistochemistry. Slices that had been incubated for up to 120 h were either snap-frozen in isopentane cooled in liquid nitrogen or fixed in 10% formalin. Frozen cryostat sections (6 μm thick) were picked up on charged slides, placed on a Ventana 320 automated immunohistochemistry stainer. The frozen sections were then reacted to the basal cell-specific murine monoclonal antibody KA2 (reactive with cytokeratins 5 and 14). Paraffin-embedded tissue was used for antibody specific to PSA, or the proliferation marker MIB-1 (reactive with Ki-67). Primary antibodies were detected using biotinylated secondary antibodies, followed by a streptavidin–peroxidase conjugate, and DAB chromogen (diaminobenzidine). Slides were counterstained with hematoxylin and coverslip mounted for examination.

Glands were graded as negative (glands with PSA expression), luminal (glands with positive PSA staining of luminal cells but negative basal cell expression), or full thickness (abnormal PSA staining, with all cells showing expression). The type of staining for each gland was determined for each slide and expressed as the mean ± standard deviation. The number of Ki-67 immunoreactive nuclei was expressed as the mean value × 100 ± standard error.

Scanning electron microscopy. Following incubation in keratinocyte basal medium supplemented with 10% fetal bovine serum, organ culture slices were obtained at 24, 48, and 72 h intervals and fixed in 1% glutaraldehyde and 4% formalin in phosphate-buffered saline. Samples were prepared for electron-microscopic evaluation by first critical point drying, then sputter coating using a gold target. The samples were evaluated and photomicrographs were obtained using a JOEL 820 scanning electron microscope.

Results

Cellular architecture

Initial experiments were performed to investigate the maintenance of organ architecture and cellular heterogeneity of precision-cut human prostate slices. Normal peripheral and transitional zone slices were prepared and incubated for up to 120 h in medium containing FBS alone or supplemented with EGF or EGF plus DHT. Histological evaluation of the slices revealed maintenance of the normal glandular structure of the organ following slicing (Figures 1, 2, 3), suggesting that the slicing process was not associated with dramatic damage. The architecture of the prostate was maintained in both peripheral and transitional zone slices incubated with keratinocyte basal medium supplemented with 10% FBS for up to 120 h (Figures 1, 2, 5, 11) or with EGF and EGF with DHT (Figure 3). Although a gradual loss of luminal cells was observed throughout the culture (Figure 7) and progressed with time in the EGF cultured slices, the basal cell population was maintained and proliferated in the presence of FBS (Figure 7). EGF medium supplemented with DHT showed reduction of loss of luminal cells (Figure 3B,D). No significant differences in either architecture or epithelial viability were noted between either the peripheral or transitional zones, or slices cultured with EGF or FBS-supplemented keratinocyte basal media.

LDH and PSA secretion

Leakage of lactate dehydrogenase has been used extensively as an indicator of cell viability in precision-cut tissue slices (Fisher et al., 1991a,b,c; Parrish et al., 1992). LDH activity in the medium, although minimal, was detected immediately following slicing; however, values remained constant from 24 h to 120 h, suggesting that minimal further cell injury occurred. DHT-supplemented medium slightly lowered the initial LDH leakage (data not shown). PSA secretion was used as an organ-specific viability indicator and was detected in the medium at all time points, although secretion decreased slightly over the course of the incubation (Figure 4). There was no significant difference in PSA secretion of slices cultured with or without DHT supplementation. Consistent with histological evaluation, significant differences in PSA secretion were not noted between peripheral or transitional zones, or in slices cultured with EGF or FBS-supplemented keratinocyte basal media.

Tissue PSA secretion

Immunohistochemical analysis was performed to assess the maintenance of cellular viability of the luminal and basal epithelium in prostate slices. Over 72 h, a loss of PSA-positive luminal cells was observed in transitional zone slices cultured with FBS-supplemented medium, lacking DHT supplementation (Figure 5). However, PSA-positive cells were maintained for up to 120 h when the EGF medium was supplemented with DHT (Figure 8). In the FBS medium-supplemented experiments, there was a loss of glands showing luminal PSA expression reaching 58% on day 2. On the third day of culture this value was

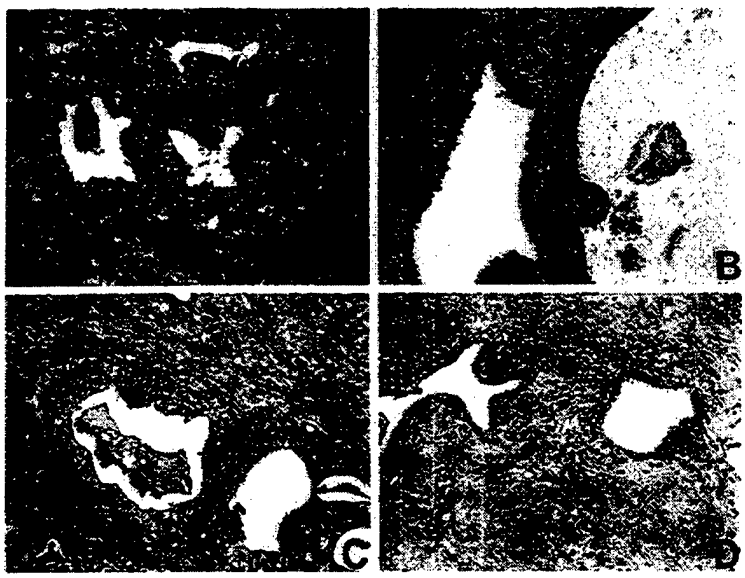

Figure 1. Histological evaluation of precision-cut human peripheral zone prostate slices in culture. Peripheral zone slices (6 mm diameter, 275–300 μm thick) were prepared from surgical tissue. The slices were placed into a dynamic organ culture system in keratinocyte basal medium supplemented with 10% FBS and maintained for up to 72 h. Slices were fixed in formalin and paraffin-embedded. Thin sections (5–7 μm) were stained with hematoxylin and eosin. A, 0 h; B, 24 h; C, 48 h; D, 72 h (original magnification ×240).

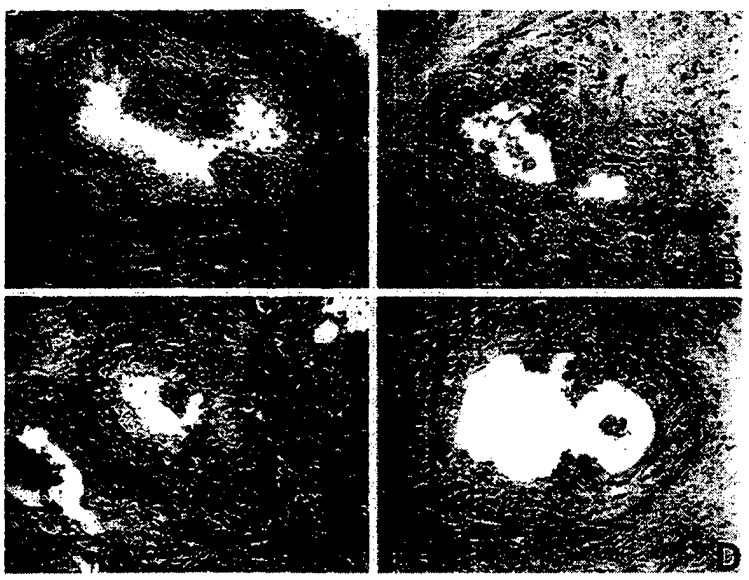

Figure 2. Histological evaluation of precision-cut human transitional zone prostate slices in culture. Transitional zone slices (6 mm diameter, 275–300 μm thick) were prepared from surgical tissue. The slices were placed into a dynamic organ culture system in keratinocyte basal medium supplemented with 10% FBS and maintained for up to 72 h. Slices were fixed in formalin and paraffin-embedded. Thin sections (5–7 μm) were stained with hematoxylin and eosin. A, 0; B, 24 h; C, 48 h; D, 72 h (original magnification ×240).

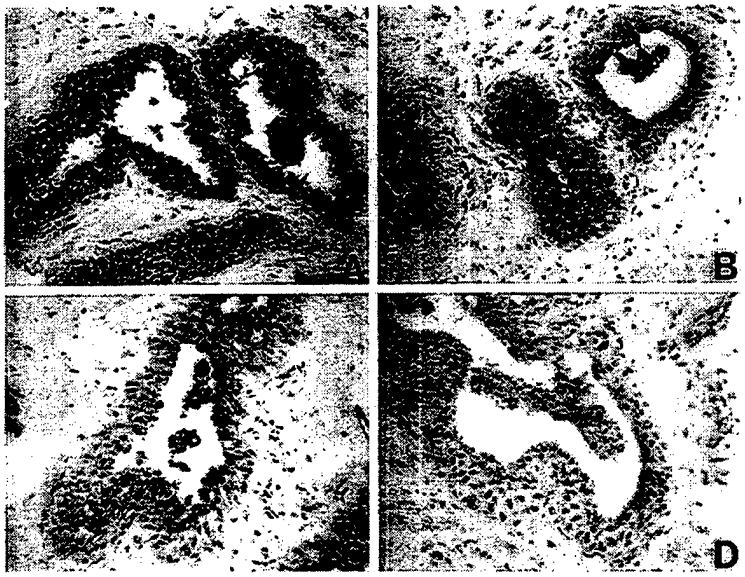

Figure 5. Immunohistochemical detection of PSA from precision-cut human prostate slices incubated up to 72 h. Transitional zone slices (6 mm diameter, 275–300 μm thick) were prepared from surgical tissue. The slices were placed into a dynamic organ culture system in keratinocyte basal medium supplemented with 10% FBS and maintained for up to 72 h. At various time points, the slices were harvested and sections were prepared. Immunohistochemistry for PSA was performed as described. A, 0 h; B, 24 h; C, 48 h; D, 72 h (original magnification ×240).

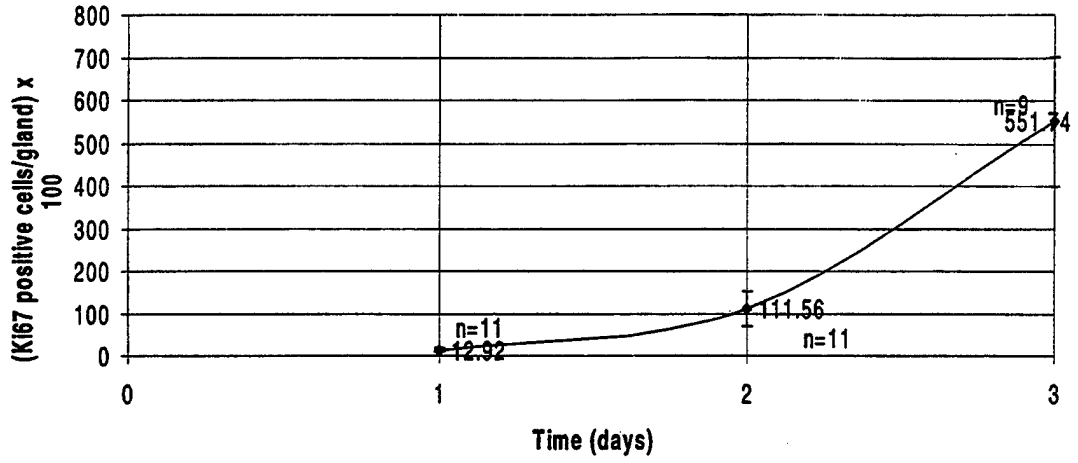

Figure 6. Basal cell proliferation versus time in organ culture. Organ culture slices from five prostates were maintained in keratinocyte medium supplemented with 10% fetal bovine serum for 1, 2, or 3 days. Slices were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 6 μm. Sections were reacted with the MIB-1 anti-Ki-67 primary antibody followed by biotinylated secondary antibodies and developed using a streptavidin–peroxidase conjugate and DAB chromogen. *n* equals the total number of slices studied. The number of positively reacting muclei were counted for each gland. Data are plotted as the mean value ×100±standard error ($n=9$).

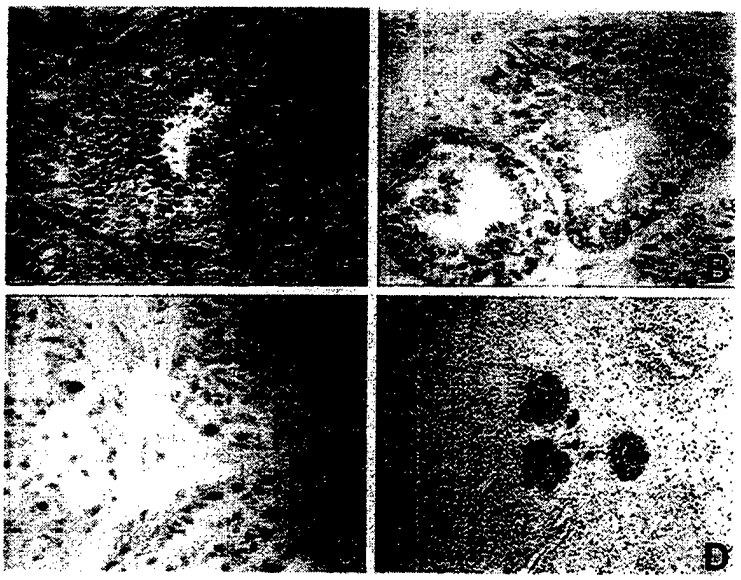

Figure 9. Five-day prostate organ cultures derived from peripheral zone. Tissue slices were cultured in the presence of keratinocyte basal medium supplemented with 10% FBS. (A) H&E showing proliferation of basal cells and preservation of luminal cells. (B) Glands reacted with basal cell specific antibodies to cytokeratins 5 and 14. Note the increased numbers of basal cells. (C) Glands reacted with MIB-1 antibody specific for Ki-67. Note proliferating cells with positive nuclear staining. (D) Prostate-specific antigen (PSA) staining. Note the emergence of three proliferating glands that definitely express PSA. Magnification: A, B, C (original magnification × 240) and D (original magnification × 75).

Figure 10. Epithelial outgrowth of a precision-cut human prostate slice. (A) Slice cultured 48 h in keratinocyte basal medium supplemented with 10% FBS. Note the outgrowth of basal cells onto cut stromal surface. Hematoxylin and eosin (original magnification × 125). (B) Scanning electron micrograph of a similar specimen showing circumferential outgrowth (arrow) onto the slice surface of epithelium arising from a gland opening (GO) (original magnification × 300).

69%. Interestingly, some glands cultured in medium containing FBS without androgen supplementation began to express PSA cytoplasmic reactivity (Figures 8 and 9D), including the proliferating cell population reaching 13% by the third day. This focal re-expression of PSA apparently does not have the same androgen dependence as the majority of the luminal cells in the prostate.

Proliferation of basal cells

Immunostaining for basal cell-specific cytokeratins in slices cultured with FBS demonstrated that the basal cells were maintained in culture (Figure 7). As expected, staining of a single layer of cells was observed in freshly cut slices. Interestingly, the basal cell layer proliferated dramatically in the glands, becoming 3–4 cell layers thick by 72 h. This was associated with increased Ki-67 staining (Figure 6). At five days, FBS-treated slices continued to show basal cell proliferation as well as viable luminal cells (Figure 9). At the surface where the glands had been cut, proliferating basal cells spread out from the gland onto the stromal surface (Figure 10). Similar results were seen for peripheral zone slices.

Cadmium-treated prostate tissue slices

Prostate tissue slices incubated for 1–3 days with 10 μmol/L $CdCl_2$ revealed a gradual increase in basal cell proliferation (H&E staining) over the 3-day period (Figure 11b). This proliferation paralleled that seen in control (untreated) tissue slices. PSA luminal staining gradually decreased over the 3-day period similarly to that of control (data not shown). However, PSA full-thickness staining, i.e., glandular cell populations that produce PSA throughout the entire colony, gradually increased over the same 3-day period.

Prostate slices incubated with 100 μmol/L $CdCl_2$ over a 3-day period manifested a dramatic increase in basal cell proliferation relative to the lower dose of $CdCl_2$ at 3 days (Figure 11C). Ki-67 staining was also increased significantly. PSA luminal staining at this dose revealed no change or a slight increase over the 3 days. PSA full-thickness staining did not change appreciably.

The prostate tissue slices treated with 500 μmol/L $CdCl_2$ demonstrated some interesting changes during the incubation period. Proliferation was enhanced in general and was as prevalent at day 1 as it was at day 3 (Figure 11D). This proliferation was accompanied by increased Ki-67 staining. However, in one patient this dose caused approximately 80% tissue necrosis by day 2. PSA luminal staining was erratic and was increased at day 3 in most patients but diminished in one patient. Several areas of marked dysplasia were noted only at this dose and only at the day 3 time point. PSA full-thickness staining was not detected at this dose.

Administration of $CdCl_2$ at concentrations of 1 mmol/L and higher produced global tissue necrosis that began immediately at day 1, with slight increase at day 3 (Figure 12B).

Discussion

Organ culture of the prostate has been proposed as an *in vitro* model to investigate prostate pathobiology since the 1970s. More recently, prostate explants have been maintained on collagen matrices (Geller et al., 1992, 1997; Olbina et al., 1998). Several types of tissue explants have been attempted including, most recently, 1 mm tissue cubes (Varani et al., 1999). The current studies were performed to establish the efficacy of slicing and maintaining precision-cut prostate tissue sections in a dynamic organ culture system with alternating exposure to ambient gases and culture media.

By producing very thin (275–300 μm) slices that are of highly reproducible dimensions, and using a dynamic organ culture system in which the tissue is not continuously submerged, the precision-cut tissue slice system represents an advance over traditional prostate organ culture. Given the retention of stromal–epithelial interactions, the ability to investigate zone-specific features, and maintenance of cellular viability and function for several days, prostate slices represent a unique

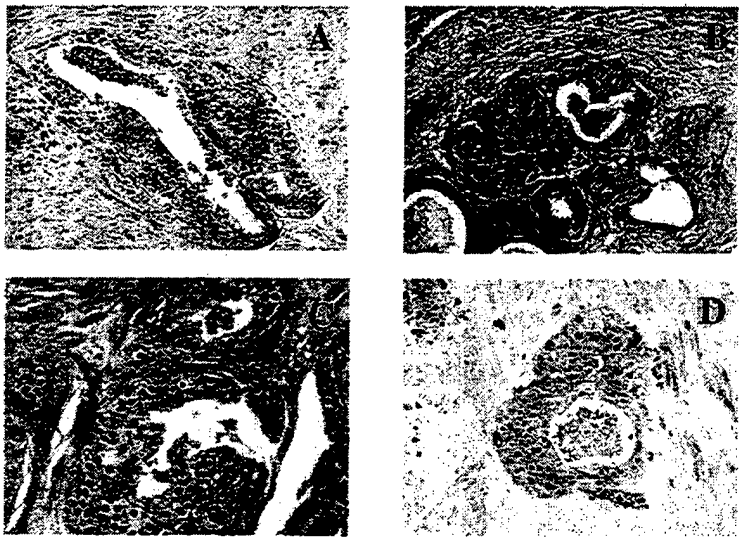

Figure 11. Comparison of control and cadmium-treated slices at three days of culture. (A) Control (untreated) slices incubated 3 days showing modest basal cell proliferation (original magnification ×200). (B) Prostate slice exposed to 10 μmol/L $CdCl_2$ for 3 days (original magnification ×200). (C) Prostate slice exposed to 100 μmol/L $CdCl_2$ for 3 days, note increased basal cell proliferation (original magnification × 400). (D) Prostate slice exposed to 500 μmol/L $CdCl_2$ for 3 days (original magnification × 200).

Figure 12. Comparison of prostate slices: control and high-dose cadmium exposure. (A) Control (untreated) prostate tissue showing modest basal cell proliferation, at 3 days of culture (original magnification × 200). (B) Prostate slice exposed to 1 mmol/L $CdCl_2$ for 3 days. Note total necrosis of glandular epithelium (original magnification × 200).

in vitro model for investigating prostate cellular proliferation and cytotoxicity.

Early studies of prostate slices using traditional submersion culture techniques showed a loss of stromal cells as early as 72 h of culturing, as well as an outgrowth of epithelial cells that were eventually overgrown by fibroblasts (Stonington and Hemmingsen, 1971). Under our dynamic organ culture system, in which tissue sections are intermittently submerged in medium and exposed to the ambient gases in a slowly rotating vial, both

stromal cells and basal cells remained viable for up to five days. In addition, the basal cell layer proliferated from a single layer of cells to a 3–4 cell layer over the five days of culture. This was associated with increased Ki-67 staining. This response appeared to require growth factors contained in the fetal bovine serum, since in the absence of FBS there was no proliferation. EGF alone was insufficient for this response. No differences were observed in the proliferative response between peripheral or transitional zone slices to the slicing/culture procedure. In this system we did not observe fibroblast overgrowth.

The finding that luminal epithelial cells were not maintained as well as basal cells was not surprising for the slices that were incubated without androgen supplementation. Secretory cells require androgens for differentiated function (Cunha et al., 1987), as evidenced by the decrease in PSA secretion throughout the incubation period. Basal cells, however, are not directly dependent upon androgens for survival (English et al., 1987; Isaacs and Coffey, 1989), although they may be androgen-responsive based on factors released from androgen-responsive stromal cells (Bonkhoff and Remberger, 1993; Leav et al., 1996). Androgen deprivation in mice is associated with a dramatic decrease in EGF in the prostate, and replacement of testosterone stimulates prostate growth and restores tissue levels of this growth factor (Hiaramatsu et al., 1988). However, EGF supplementation alone did not maintain the luminal cells in the precision-cut slices. DHT was necessary to maintain viability and function of the luminal cells in prostate slices. The remarkable proliferation of the basal cell compartment, stimulated by a component of FBS, offers the possibility of studying the regulation of basal cell growth in this organ. Interestingly, the addition of DHT did not enhance this response.

Cadmium (Cd), a known toxin and human carcinogen, has been implicated as a prostate carcinogen, although the mechanisms are unknown. Achanzar et al. (2001) recently described the *in vitro* malignant transformation of RWPE-1 cells exposed to 10 μmol/L $CdCl_2$ and Ye et al. (2000) reported androgen-like activity of Cd in the *in vitro* activation of the androgen response element. We present evidence here that Cd has a bimodal effect on prostate tissue. At lower concentrations (10–100 μmol/L), Cd promotes basal cell proliferation. At higher concentrations, there are effects ranging from increased proliferation and dysplasia of the basal cells at 500 μmol/L to cytotoxicity and necrosis at concentrations above 500 μmol/L.

Another tissue culture system similar to ours was described recently (Varani et al., 1999), with several comparable results seen between that study and ours. These investigators, using 1 mm cubes of human prostate, also noted that the central tissue architectural features of stroma and epithelium were maintained over four days. They observed a similar gradual loss of secretory cells after four days. We have confirmed the finding that the basal cell population was maintained and proliferated. The incubations were not affected by any fibroblastic outgrowth that frequently occurs with prostate cell culture. We also had an absence of fibroblastic outgrowth problems with our system.

Although the system used by Varani et al. yielded comparable results to ours, we feel that there are several distinct advantages of our dynamic organ culture system. The tissue slices are very reproducible. In addition, we have the capability of generating "sister" slices, i.e., consecutive slices to be used for purposes of direct comparison. The slices have the added benefit of a large surface area, which provides a broader field of adjacent glandular structures. The thinness of slices and the dynamic incubation permit better tissue

exchange of nutrients, gases, hormones, and growth factors from the medium. The tissue cores acquired at the time of prostatectomy are readily identified as transitional or peripheral zone samples. This system also affords the unique opportunity of evaluating the changes of protein expression in response to various factors that can be added to the medium, e.g., PSA which can be measured not only in the medium but also in the cells. In conclusion, precision-cut human peripheral or transitional prostate slices maintain important features of the prostate, including organ architecture and cellular heterogeneity.

In the future, this dynamic organ culture system can be applied to study the stromal–epithelial interactions of the prostate gland in response to the effects of hormones and growth factors. We also feel that an exciting application will be in the use of this system to study site-specific effects of drugs on the prostate. This would be particularly useful when targeting selected cells such as those that secrete PSA.

Acknowledgments

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